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Frequency of IRF5+ dendritic cells is associated with the TLR7-induced inflammatory cytokine response in SARS-CoV-2 infection

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ABSTRACT

The SARS-CoV-2 infection leads to enhanced inflammation driven by innate immune responses. Upon TLR7 stimulation, dendritic cells (DC) mediate the production of inflammatory cytokines, and in particular of type I interferons (IFN). Especially in DCs, IRF5 is a key transcription factor that regulates pathogen-induced immune responses via activation of the MyD88-dependent TLR signaling pathway. In the current study, the frequencies of IRF5+ DCs and the association with innate cytokine responses in SARS-CoV-2 infected individuals with different disease courses were investigated. In addition to a decreased number of mDC and pDC subsets, we could show reduced relative IRF5+ frequencies in mDCs of SARS-CoV-2 infected individuals compared with healthy donors. Functionally, mDCs of COVID-19 patients produced lower levels of IL-6 in response to *in vitro* TLR7 stimulation. IRF5+ mDCs more frequently produced IL-6 and TNF-α compared to their IRF5− counterparts upon TLR7 ligation. The correlation of IRF5+ mDCs with the frequencies of IL-6 and TNF-α producing mDCs were indicators for a role of IRF5 in the regulation of cytokine responses in mDCs. In conclusion, our data provide further insights into the underlying mechanisms of TLR7-dependent immune dysfunction and identify IRF5 as a potential immunomodulatory target in SARS-CoV-2 infection.

1. Introduction

Coronavirus disease 2019 (COVID-19) caused by infection with the novel severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), presents with a wide range of clinical manifestations, with most patients experiencing a mild to moderate course, while others progress to severe or critical disease [1]. The underlying causes of these different clinical phenotypes are not yet fully understood. A potential pathophysiological mechanism may be a dysregulation of inflammatory responses, leading to a so-called "cytokine storm" [2–7].

Dendritic cells (DCs) play a crucial role in the innate immune response against viral infections. RNA viruses, such as SARS-CoV-2, are sensed by DCs via Toll-like receptors (TLRs), including TLR7, resulting in the production of innate and inflammatory cytokines [8]. In humans, at least two distinct DC subsets, (HLA-DR+CD11c−CD123+) plasmacytoid DCs (pDCs) and (HLA-DR+CD11c+CD123−) myeloid DCs (mDCs), have been identified and are known to respond to different TLR ligands [9]. Some studies have shown a decreased number of

Abbreviations: COVID-19, Coronavirus Disease 2019; CRP, C-reactive protein; DC, Dendritic cell; ECMO, Extracorporal membrane oxygenation; HD, Healthy donor; ICU, Intensive care unit; IRF5, Interferon regulatory factor 5; IFN-α, Interferon alpha; IL-6, Interleukin 6; ILT-3, Immunoglobulin-like transcript 3; MFI, Median fluorescence intensity; mDC, Myeloid Dendritic Cell; MyD88, Myeloid differentiation primary response 88; PBMC, Peripheral Blood Mononuclear Cell; PCR, Polymerase chain reaction; pDC, Plasmacytoid Dendritic Cell; SARS-CoV-2, Severe acute respiratory syndrome coronavirus type 2; SNP, single-nucleotide polymorphism; TLR7, Toll-like receptor 7; TNF-α, Tumor necrosis factor alpha.

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plasmacytoid and myeloid DCs in the peripheral blood of patients with acute SARS-CoV-2 infection and described an association with disease severity [10,11]. Increasing data suggest an important role of the TLR7 pathway and its resulting cytokine production in the outcome of COVID-19 [12,13]. A better understanding of the pathways downstream of TLR7 signaling is an essential step in the design of new therapeutic targets to modulate harmful immune responses in COVID-19. One potential target of interest that is known to play a critical role in responses through the TLR - myeloid differentiation primary response 88 (MyD88) pathway is the Interferon regulatory factor (IRF) 5 [14]. After viral sensing, IRF5 is a key transcription factor for the activation of innate immune responses by induction of several inflammatory cytokines, including type I interferons (IFN), tumor necrosis factor-alpha (TNF-α), and Interleukin-6 (IL-6) [14–17]. So far, data about the IRF5 expression signature on DCs, especially after TLR7-dependent activation, and its functional consequences in SARS-CoV-2 infection have not been described.

In this study, the IRF5 expression pattern was analyzed on different peripheral blood dendritic cell populations in a cohort of COVID-19 patients, and the potential association of IRF5 with the impaired cytokine production pattern of patients with COVID-19 was tested. With this study, we provide further insights into underlying mechanisms of TLR7-dependent immune dysfunction and identify IRF5 as a potential immunomodulatory target in SARS-CoV-2 infection and other viral diseases.

2. Material and methods

2.1. Study population

A total number of 46 study participants were enrolled in this study between March 2020 and April 2021 at the University Medical Center Hamburg-Eppendorf, Germany. Venous whole blood samples from SARS-CoV-2 infected patients (n = 30) and uninfected healthy individuals (n = 16) were collected in Vacutainer CPT tubes (BD). All COVID-19 patients were treated as inpatients and SARS-CoV-2 infection was confirmed by polymerase chain reaction (PCR) of nasopharyngeal or oropharyngeal swab specimen as previously described [18]. Disease severity was graded by clinical presentation according to the STAKOB [19] (Robert Koch Institute, Berlin, Germany; classification adapted from *WHO Therapeutics and COVID19: living guideline*, see also Supplementary Table S1). All participants gave written informed consent. The study was approved by the local ethics board of the *Ärztekammer Hamburg* (PV4780, PV7298).

2.2. Sample processing and stimulation

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation and cryopreserved in Roswell Park Memorial Institute medium (RPMI1640, Gibco) supplemented with 25% heat-inactivated fetal calf serum (FCS) and 10% dimethylsulfoxide. Cryopreserved PBMCs were thawed as needed and either directly stained for surface markers and intranuclear IRF5 or stimulated as previously described [20]. In short, samples were stimulated with 1μg/ml of the synthetic TLR7 agonist CL097 (Sigma-Aldrich) for 20 h at 37 °C and 5% CO_2 in RPMI1640 supplemented with 10% heat-inactivated FCS, 1% penicillin/streptomycin, and 1% HEPES (Gibco). To inhibit the cytokine secretion, Brefeldin A (Sigma-Aldrich) at a final concentration of 5μg/ml was added after one hour.

2.3. Intracellular staining of IRF5 protein and flow cytometric analysis

PBMCs were stained for surface markers and intranuclear IRF5. The cells were washed and stained with LIVE/DEAD fixable Near-IR dead cell stain (Invitrogen) and fluorochrome-conjugated surface antibodies (see Supplementary Table S2). Cells were fixed using 1% paraformaldehyde and subsequently permeabilized with 0.1% Triton X-100. Fixed cells were first incubated with an anti-IRF5 antibody (E7F9W, Cell Signaling Technology) for 20 min and then a secondary anti-rabbit-IgG AlexaFluor488 antibody (Cell Signaling Technology) for 15 min. Stimulated samples and unstimulated controls were stained for intracellular cytokines with fluorochrome-labeled antibodies. Samples were acquired within 12 h on a BD LSRFortessa II (BD Biosciences). Cells were analyzed using FlowJo 10.7 software (BD Biosciences) with the exclusion of doublet cells. mDCs were identified as LD−, CD3−, CD14−, CD19−, CD20−, HLA-DR+, CD11c+, and CD123− cells. pDCs were identified as LD−, CD3−, CD14−, CD19−, CD20−, HLA-DR+, CD11c−, and CD123+ cells. Background staining was assessed for every sample by staining without the primary anti-IRF5 antibody (isotype control).

2.4. Assessment of antibody specificity

IRF5 expression is mainly restricted to DCs and B cells [17,21,22]. To confirm anti-IRF5 antibody specificity, the expression of IRF5 in CD3+ T cells was identified with the aid of isotype controls (see Supplementary Fig. S1). In line with the literature [23], antibody specificity was considered to be reliable as CD3+ T cells did not express IRF5.

2.5. Statistical analyses

GraphPad Prism v7 (GraphPad Software) was used to analyze the data. Mann-Whitney *U* tests and Wilcoxon matched-rank tests were used to determine statistical significance between unpaired and paired data sets, respectively. Spearman's rank correlation coefficient was calculated to determine the correlation between different data sets. A *P*-value < 0.05 was considered significant.

3. Results

3.1. Clinical characteristics of patients with SARS-CoV-2 infection

30 individuals hospitalized with acute SARS-CoV-2 infection were recruited at the University Medical Center Hamburg-Eppendorf between March 2020 and April 2021 (Table 1). 16 healthy, uninfected individuals served as a control group. Infected patients had different clinical manifestations showing a moderate (n = 13), severe (n = 11), or critical (n = 6) course of COVID-19. The patients were enrolled within 20 days (mean 5.5 days) after they tested positive for SARS-CoV-2 by PCR from nasopharyngeal or oropharyngeal swab specimens.

Moderately ill patients had a mean age of 54 years (range: 36–86) and thus were slightly younger than the patients with severe (mean 64 years; range: 52–75) or patients with a critical course (mean 64 years; range: 52–75). Sex ratios were balanced in all groups. Chronic diseases were more common among severely and critically ill patients (n = 17/17; 100%) compared to the moderately ill ones (n = 11/13; 84.6%). The most common comorbidities were hypertension (n = 12/30; 40.0%) and diabetes (n = 11/30; 36.7%). All patients with a severe or critical course of disease (n = 17/17; 100%) required oxygen supplementation, while only 30.8% (n = 4/13) of patients with moderate disease had oxygen administered. In all groups, a certain proportion of patients were admitted to the intensive care unit (ICU). Of the individuals with moderate COVID-19, 15.4% (n = 2/13) were admitted to the ICU, but no invasive ventilation or extracorporeal membrane oxygenation (ECMO) was necessary. However, 54.5% (n = 6/11) of severely infected and 100% (n = 6/6) of critically infected patients required a stay at the ICU with 9.1% (n = 1/11) and 66.7% (n = 4/6) requiring intubation, respectively. ECMO was established in two patients (33.3%) with critical COVID-19. Certain inflammation markers such as C-reactive protein (CRP), Interleukin-6 (IL-6), Procalcitonin, and Ferritin were extracted from the clinical database. Expectedly, mean values for all of these parameters were higher with increasing disease severity. Levels of IL-6 were highest in critically ill patients compared to those with a severe

Table 1
Clinical characteristics of patients with acute SARS-CoV-2 infection and healthy donors. Values are given as absolute numbers with percentages or mean with range. Time since diagnosis refers to the time passed since the first positive PCR swab. Laboratory results are derived from the clinical database from the day of the sampling ± 2 days. Abbreviations: n/a, not applicable; ECMO, extracorporeal membrane oxygenation; CRP, C-reactive protein; IL-6, Interleukin-6. Reference values of laboratory results: CRP < 5 mg/dl; IL-6 < 7 ng/l; Procalcitonin < 0.5 µg/l; Ferritin 10–291 µg/l; D-dimer 0.21–0.52 mg/l.

	COVID-19			Healthy donors
	Moderate n = 13	Severe n = 11	Critical n = 6	n = 16
Age in years, mean (range)	54 (36-86)	64 (52–75)	64 (52–75)	37 (21–66)
Sex at birth, n (%)				
Female	7 (53.8%)	5 (45.5%)	3 (50%)	8 (50%)
Male	6 (46.2%)	6 (54.5%)	3 (50%)	8 (50%)
Days since diagnosis, mean (range)	3.9 (1–10)	7.4 (2–20)	5.7 (1–15)	n/a
Comorbidities, n (%)				
None	2 (15.4%)	–	–	16 (100%)
Hypertension	5 (38.5%)	4 (36.4%)	3 (50%)	–
Diabetes	3 (23.1%)	4 (36.4%)	4 (66.7%)	–
Heart diseases	4 (30.8%)	2 (18.2%)	2 (33.3%)	–
Lung diseases	1 (7.7%)	4 (36.4%)	1 (16.7%)	–
Cancer	1 (7.7%)	–	2 (33.3%)	–
Other	9 (69.2%)	5 (45.5%)	5 (83.3%)	–
Intensive Care Unit, n (%)	2 (15.4%)	6 (54.5%)	6 (100%)	n/a
Invasive ventilation	–	1 (9.1%)	4 (66.7%)	
ECMO	–	–	2 (33.3%)	
Oxygen supplementation, n (%)	4 (30.8%)	11 (100%)	6 (100%)	n/a
Laboratory results, mean (range)				n/a
CRP [mg/l]	29 (<4-72)	73 (11–202)	140.7 (52–207)	
IL-6 [ng/l]	17.4 (<1.5–49.8)	55.3 (<1.5–181.2)	92.5 (14.6–230.5)	
Procalcitonin [µg/l][†]	0.04 (<0.02–0.25)	0.09 (0.02–0.18)	0.37 (0.06–1.04)	
Ferritin [µg/l]	409.4 (6.8–1503.3)	568.1 (74.7–1249.2)	693.2 (111.6–1948.7)	
D-dimer [mg/l]	1.82 (<0.19–16.57)	3.68 (0.63–23.05)	1.62 (0.37–3.66)	
Patients still alive, n (%)	13 (100%)	9 (81.8%)	4 (66.7%)	n/a

[†] Procalcitonin results were only available for n = 12 and n = 10 individuals with moderate or severe COVID-19, respectively.

or a moderate course of diseases (mean IL-6: 92,5ng/l versus 55,35 ng/l and 17,45 ng/l, respectively). Elevated d-dimers, which are indicative of COVID-19 associated coagulopathy [24] and prognosis [25], did not follow this trend.

Three months after SARS-CoV-2 infection, all moderately infected individuals (n = 13/13; 100%), 81.8% (n = 9/11) of severely infected, and 66.7% (n = 4/6) of critically infected patients were still alive.

3.2. *Patients with SARS-CoV-2 infection show reduced relative frequencies of dendritic cell subsets*

It has been described that the frequencies of all dendritic cell subtypes are reduced in the peripheral blood of COVID-19 patients [10,11,26]. Therefore, we first aimed to confirm this finding in our cohort. The frequency of Lin-HLA-DR+CD123−CD11c+mDCs and Lin-HLA-DR+CD123+CD11c−pDCs (Fig. 1A) was determined of single lymphocytes in the PBMCs of COVID-19 patients and compared to healthy donors. Indeed, the patients with acute SARS-CoV-2 infection exhibited significantly lower frequencies of both pDCs (mean 0.31% vs 0.54%, $P = 0.0178$) and mDCs (mean 0.62% vs 1.40%, $P = 0.0438$) compared with healthy individuals (Fig. 1B). These observations suggest a suppression of mDC and pDC populations in patients with acute SARS-CoV-2 infection.

In DCs, IRF5 is involved downstream of the TLR7 signaling pathway as a master transcription factor in the activation of genes for inflammatory cytokines [27]. Thus, potential alterations in the expression of IRF5 could contribute to the COVID-19 pathogenesis. As assessed by flow cytometry, there was no difference of IRF5+ pDCs (mean 54.38% vs 62.4%, $P = 0.9094$) or the IRF5 MFI in pDCs ($P = 0.9801$) between COVID-19 patients and healthy donors (Fig. 1C). The extremely broad range of the IRF5+ frequency could not be satisfactorily explained by any clinical parameters but could be the result of single nucleotide polymorphisms (SNPs) in the IRF5 gene influencing its expression [28]. However, the mDCs of COVID-19 patients less frequently expressed IRF5 (81.36% vs 97.46%, $P < 0.0001$), and the IRF5 MFI also tended to be lower ($P = 0.0571$) compared to healthy controls (Fig. 1D). Age, sex, and disease severity were not associated with a decrease in any of the DC populations or their IRF5 expression (Supplementary Figs. S2-S4). Of note, the IRF5 MFI was significantly higher in mDCs than in pDCs in both the samples of COVID-19 patients and healthy donors (Fig. 1E). This suggests that IRF5 might be of greater importance in the regulation of mDCs than pDCs. Taken together, acute SARS-CoV-2 infection was associated with a profound alteration of the dendritic cell compartment, particularly the decrease of DC subsets and reduced IRF5 expression in mDCs.

3.3. *Preserved pDC function but reduced IL-6+ mDCs after ex vivo TLR7 stimulation in patients with acute SARS-CoV-2 infection*

TLR7 sensing and signaling are important for the recognition of SARS-CoV-2 and the initiation of innate immune responses [8,13]. To assess the impact of TLR7 signaling on the IRF5 expression and cytokine production in the DC populations, PBMCs of COVID-19 patients and healthy donors were stimulated with a synthetic TLR7 agonist (CL097) [20]. The cytokine production and IRF5 expression were measured by flow cytometry.

Upon stimulation, the frequency of IRF5+ pDCs was significantly increased in both patients with acute SARS-CoV-2 infection ($P = 0.0078$) and healthy individuals ($P = 0.0078$; Fig. 2A). While the frequency of IRF5+ pDCs following TLR7 stimulation was comparable between COVID-19 patients and healthy donors (mean 68.3% vs 58.8%, $P = 0.3213$), healthy individuals tended to more strongly upregulate IRF5 according to the MFI (Fig. 2A), although this did not reach statistical significance ($P = 0.0592$). However, the pDCs activation as measured by the upregulation of HLA-DR (Fig. 2B) was comparable between COVID-19 patients and healthy donors and the proportion of IL-6, TNF-α or IFN-α producing pDCs did not differ either (Fig. 2C). Of note, the production of TNF-α but not the other cytokines were negatively associated with patient age (Supplementary Fig. S5). These results suggest preserved functionality in the remaining pDCs of COVID-19 patients regarding the

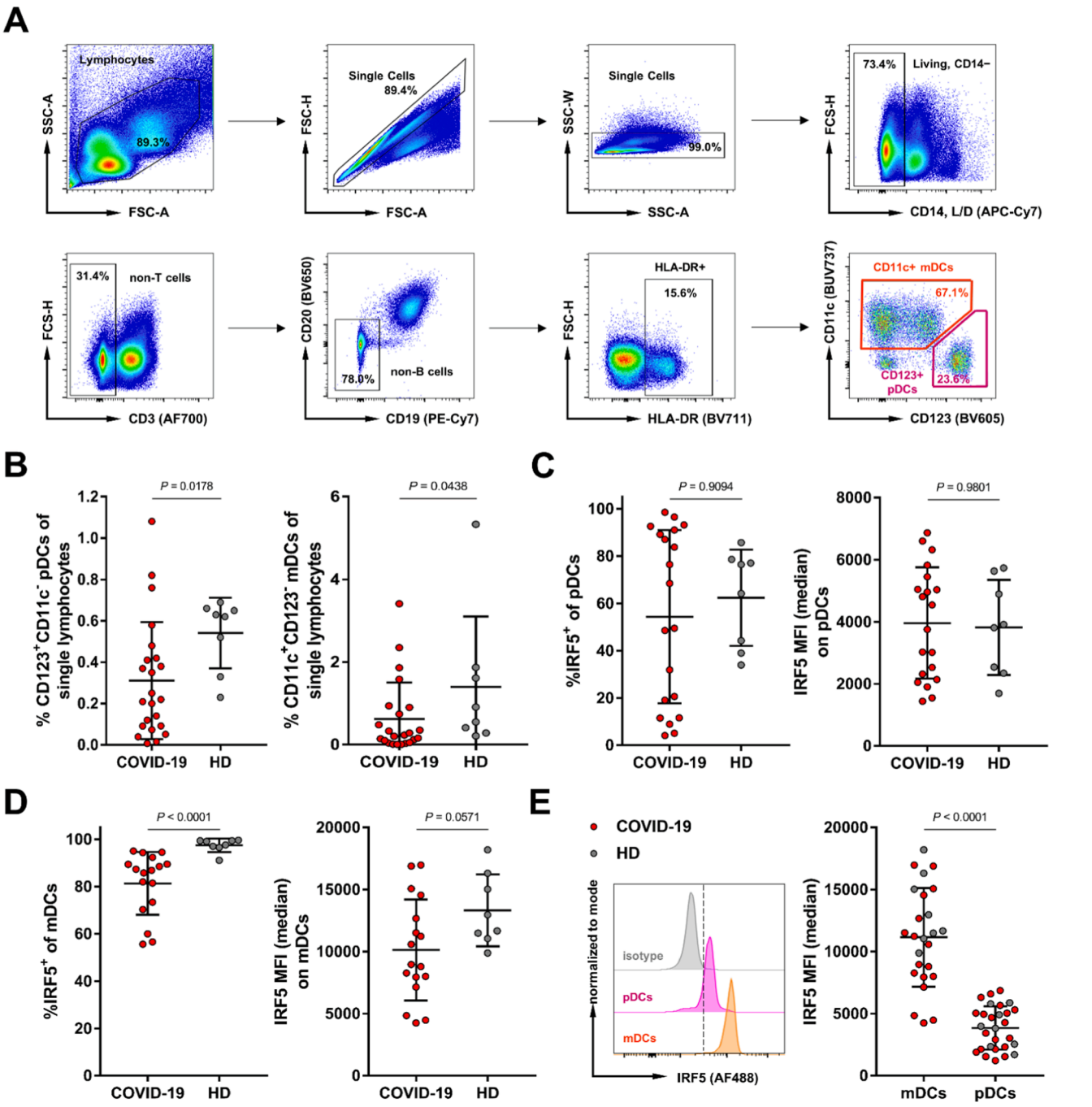

Fig. 1. SARS-CoV-2 infection is associated with profound alterations of the dendritic cell compartment. (**A**) PBMCs of COVID-19 patients and healthy donors (HD) were analyzed by flow cytometry to assess the blood dendritic cell compartment. Plasmacytoid dendritic cells (pDCs) were identified as single, viable, Lin−, HLA-DR+, CD11c−, CD123+ cells (pink), and myeloid dendritic cells (mDCs) as single, viable, Lin−, HLA-DR+, CD11c+, CD123− cells (orange). (**B**) The frequencies of both pDCs and mDCs of single lymphocytes were significantly reduced in patients with COVID-19 compared with healthy controls. (**C**) While the pDCs of SARS-CoV-2 infected patients showed comparable frequencies of IRF5+ cells and a similar IRF5 MFI compared with healthy donors, (**D**) IRF5+ mDCs were significantly reduced and the IRF5 MFI also tended to be lower in mDCs of COVID-19 patients. (**E**) IRF5 expression was significantly higher in mDCs compared to pDCs. For all analyses, a Mann-Whitney *U* test was performed to assess the statistical significance. Red dots indicate individuals with acute SARS-CoV-2 infection and grey dots show healthy donors.

investigated parameters despite the strong suppression of the pDC populations.

The mDCs responded somewhat differently to the CL097 stimulus. The proportion of IRF5+ mDCs did not increase significantly upon TLR7 ligation (Fig. 3A), neither for the COVID-19 patients ($P = 0.8311$) nor the healthy donors ($P = 0.5469$). However, consistent with the data in pDCs, the mDCs of patients with acute COVID-19 seemed to have a reduced capacity to upregulate IRF5 according to the MFI change although this was not statistically significant ($P = 0.0908$; Fig. 3A). Furthermore, the upregulation of HLA-DR as an indicator of DC activation was comparable between COVID-19 patients and healthy donors (Fig. 3B). To further analyze the functionality of the mDCs in SARS-CoV-2 infection, the percentage of IL-6 and TNF-α producing mDCs was determined by flow cytometry after TLR7 stimulation (Fig. 3C). Compared with healthy individuals, mDCs of COVID-19 patients had significantly lower levels of IL-6 producing mDCs ($P = 0.0231$). The

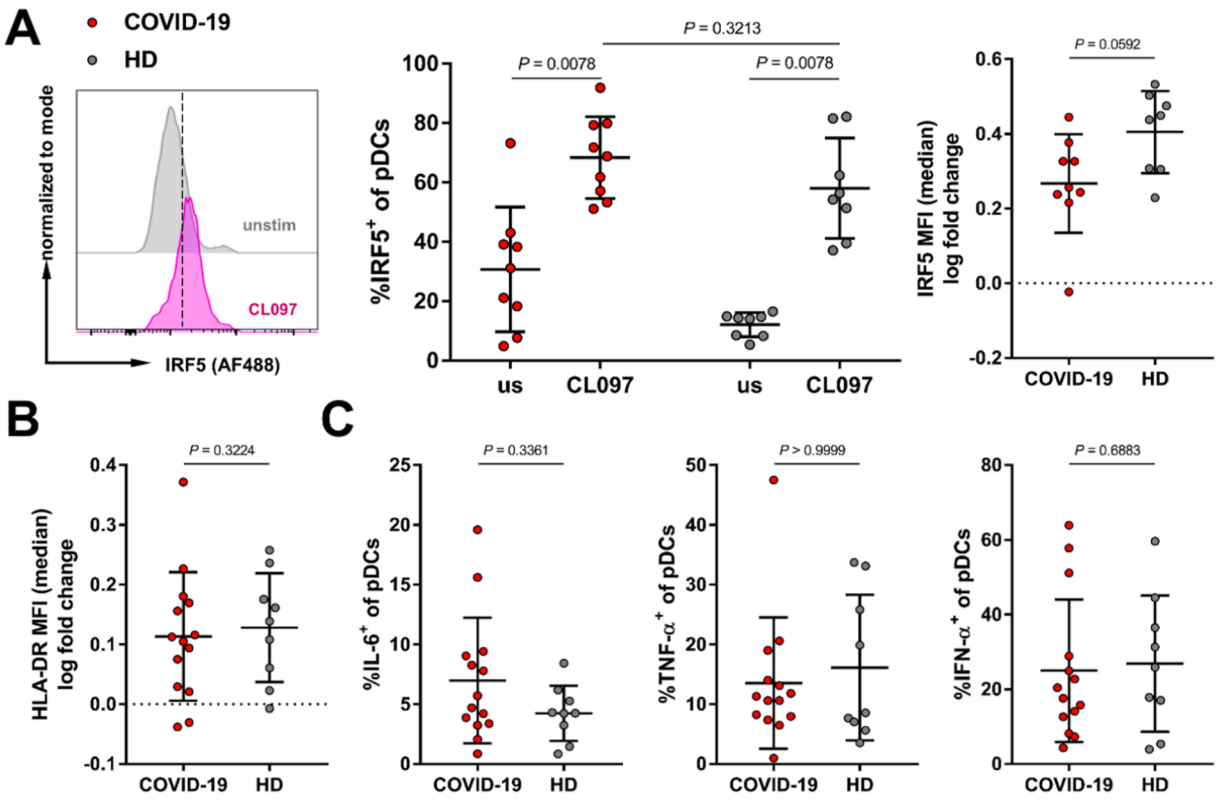

Fig. 2. pDCs upregulate IRF5 upon TLR7 ligation. (**A**) pDCs upregulate IRF5 similarly upon TLR7 engagement in both COVID-19 patients and healthy donors. (**B**) Comparable upregulation of HLA-DR on pDCs upon TLR7 ligation between COVID-19 patients and healthy controls. (**C**) No significant alterations between COVID-19 patients and healthy donors in the cytokine profile of pDCs upon TLR7 ligation. For comparison of IRF5+ frequency before and after TLR7 stimulation within the COVID-19 group and the healthy donors, statistical significance was determined with a Wilcoxon matched-rank test. For all other analyses, a Mann-Whitney *U* test was performed. Red dots indicate individuals with acute SARS-CoV-2 infection and grey dots show healthy donors.

numbers of TNF-α+ mDCs were comparable with healthy donors ($P = 0.7051$) and there were no relevant numbers of IFN-α producing mDCs. There was no association between mDC responsiveness and age (Supplementary Fig. S6). These results demonstrate an impaired cytokine response of mDCs in response to TLR-7 stimulation in SARS-CoV-2 infected individuals.

3.4. IRF5+ frequency correlates with IL-6 and TNF-α producing mDCs of COVID-19 patients

To understand the role of IRF5 in the regulation of dendritic cells, the association between IRF5 expression and cytokine production was assessed. For both pDCs and mDCs, we observed higher frequencies of IL-6 and TNF-α producing cells within the IRF5+ populations compared to their IRF5− counterparts (Fig. 4A-B and Supplementary Fig. S7A-B). These differences could be observed in COVID-19 patients and healthy donors alike but was more pronounced in mDCs than pDCs. Regarding IFN-α production, there was no difference between IRF5+ and IRF5− pDCs (Supplementary Fig. S7C). Just like the higher IRF5 MFI in mDCs (Fig. 1E), these results suggest that IRF5 might be more important for the regulation of effector functions of mDCs than pDCs. We detected a correlation of IRF5+ mDCs with the cytokine-producing mDCs of COVID-19 patients which was not present for pDCs and supported these findings (Fig. 4A-B and Supplementary Fig. S7A-C). Additionally, the IRF5 MFI correlated with the IL-6 MFI in mDCs (Supplementary Fig. S8A). However, the IRF5 MFI did not correlate with the IL-6 MFI in pDCs or with the MFI of the other cytokines (Supplementary Fig. S8A-B). Interestingly, the IRF5+ populations of pDCs and mDCs also showed higher frequencies of cells expressing the inhibitory receptor CD85k (ILT-3) compared to the IFR5− populations (Fig. 4C and Supplementary Fig. S7D). Again, on mDCs we could observe a strong correlation CD85k+ and IRF5+ cells for both COVID-19 patients and healthy donors. Taken together, these results are indicators for a potential role of IRF5 as a regulator of IL-6 and TNF-α production as well as CD85k expression in mDCs.

4. Discussion

The dysregulation of innate immune responses is a feature of severe COVID-19 [26]. DC subsets play a critical role in the activation of innate immune responses: during acute viral infections, DCs sense pathogens via the nucleic acid sensor TLR7 and produce high levels of pro-inflammatory cytokines in response [29]. We aimed to investigate the association of IRF5 with the TLR7-dependent immune regulation in the DC compartment in COVID-19.

In accordance with previously published studies that characterized host immune responses in COVID-19 [10,11,26,30–32], we observed a significant decrease of the myeloid and plasmacytoid DC frequencies in the peripheral blood of infected patients independently of the disease severity. These results suggest that DCs, the main producers of type I IFNs, are impaired in COVID-19, which is consistent with previously published studies [11,31,32] and with murine SARS-CoV infection [33]. A possible explanation could be DC migration from peripheral blood to local inflammatory site tissue. Accordingly, Sanchéz-Cerrillo *et al.* described that mDCs of patients with severe COVID-19 preferentially migrate to the lungs [34].

IRF5 acts downstream of TLR7 and subsequently induces cytokine production, particularly in DCs [14–17]. IRF5 has been implicated in many inflammatory-driven diseases, such as systemic lupus erythematosus and inflammatory bowel disease [28]. So far only a few studies about the functional characteristics and role of IRF5 in COVID-19 induced immune responses have been published. In the current study,

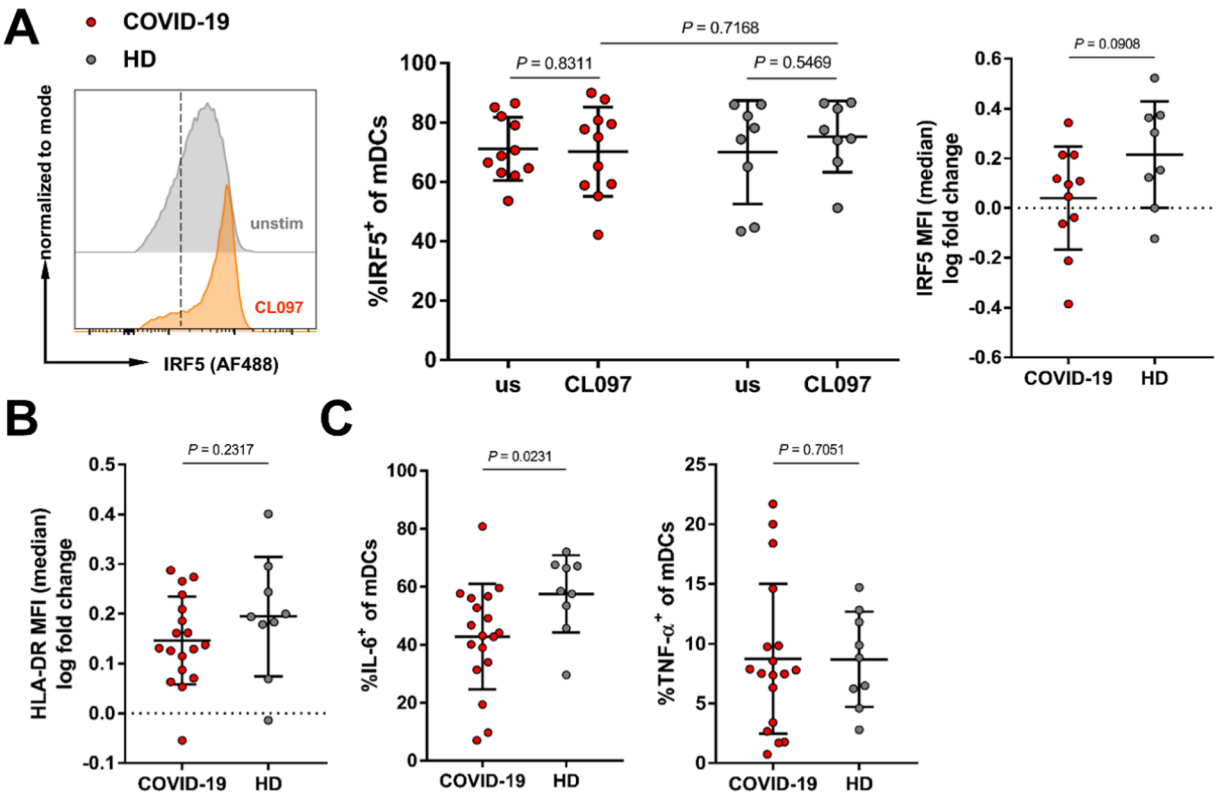

Fig. 3. Reduced frequencies of IL-6+ mDCs in patients with COVID-19. (**A**) There was no significant increase of IRF5+ mDCs upon TLR7 engagement in both COVID-19 patients and healthy donors, but a trend towards stronger upregulation of IRF5 MFI by healthy donors. (**B**) Comparable upregulation of HLA-DR on mDCs upon TLR7 ligation between COVID-19 patients and healthy controls. (**C**) No significant differences between COVID-19 patients and healthy donors with respect to TNF-α production but IL-6 production of mDCs upon TLR7 ligation. For comparison of IRF5+ frequency before and after TLR7 stimulation within the COVID-19 group and the healthy donors, statistical significance was determined with a Wilcoxon matched-rank test. For all other analyses, a Mann-Whitney *U* test was performed. Red dots indicate individuals with acute SARS-CoV-2 infection and grey dots show healthy donors.

IRF5 expression levels in DCs of infected patients and healthy individuals were analyzed to gain a better understanding of its importance in COVID-19, where it may contribute to harmful innate immune responses. Here, we could show that IRF5+ mDCs were significantly reduced but the frequency of IRF5+ pDCs was maintained in SARS-CoV-2 infected individuals compared to healthy donors, as measured by flow cytometry. While TLR7 stimulation led to a significant upregulation of IRF5+ pDCs, IRF5+ mDCs did not significantly change in response to TLR7. However, compared to healthy individuals, infected individuals still tended to have impaired IRF5 MFI upregulation following TLR7 stimulation in both pDCs and mDCs. Altogether, these results suggest that IRF5 is partially regulated by TLR7 signaling and hence is critically involved in the pathogenesis of COVID-19.

A study by Yin *et al.* reported that IRF5 is required for the IFN response induced by SARS-CoV-2 in Vero E6 cells [35]. To determine whether the reduced IRF5+ DCs are associated with impaired cytokine production and altered IFN response, PBMCs were stimulated with the TLR7 agonist CL097 [20]. Contradicting our hypothesis, the dendritic cell cytokine profiles of COVID-19 patients were comparable to the ones in healthy donors following TLR7 stimulation. Solely, TLR7-stimulated mDCs of COVID-19 patients showed an impaired capacity to produce IL-6. These data suggest that while COVID-19 causes a depletion of blood DCs, the cytokine response is largely maintained. In contrast, a previous study by Pérez-Gémez *et al.* investigating immune dysregulation in COVID-19 observed a decrease in pDC levels and a considerable reduction of IFN-α production following TLR9 stimulation in acute SARS-CoV-2 infected patients [32]. Arunachalam *et al.* showed that the IFN-α production of pDCs was impaired in SARS-CoV-2 infected patients compared to healthy individuals [31].

While we could not confirm these alterations of the innate cytokine response in COVID-19 patients after TLR7 stimulation, our study provides strong indications for a potential role of IRF5 in the regulation of IL-6 and TNF-α production in mDCs and to a smaller degree as well in pDCs. In contrast, IFN-α production of pDCs seemed to be mostly independent of the IRF5 expression. This renders IRF5 a potential therapeutic target since an inhibition could possibly reduce IL-6 and TNF-α mediated hyperinflammatory states while maintaining antiviral type I IFN activity. Of note, the inhibitory receptor CD85k had also strong associations to the IRF5 expression in both pDCs and mDCs. Although this could be interpreted as a marker of DC activation or an intrinsic regulatory mechanism to limit excessive immune responses, future studies need to address the importance of this finding.

Limited sample numbers and thus underpowered analyses restrain our results to some extent. Therefore, there is the need to investigate the role of IRF5 in larger cohorts. Also, age differences between healthy controls and COVID-19 patients might affect the interpretation of our data due to altered cytokine secretion in aged immune systems [36]. However, we did not find the age to be a confounding factor for altered IRF5 expression or cytokine production. While our results show coinciding cytokine and IRF5 expression, we cannot causally link IRF5 to DC functionality because we were not able to perform an IRF5 knockdown. Furthermore, multiple SNPs have been identified in the human IRF5 gene that alter the expression levels of IRF5 and hence influence the magnitude of host inflammatory responses and risk of severe COVID-19 [28,37], which was not considered in this study. Future studies should further differentiate the different mDC subsets, in particular CD141+ mDCs and CD1c+ mDCs to examine potential differences in the IRF5 regulation [9].

Taken together, we observed a significant decrease in the percentage of DC subsets in the peripheral blood of SARS-CoV-2 infected patients.

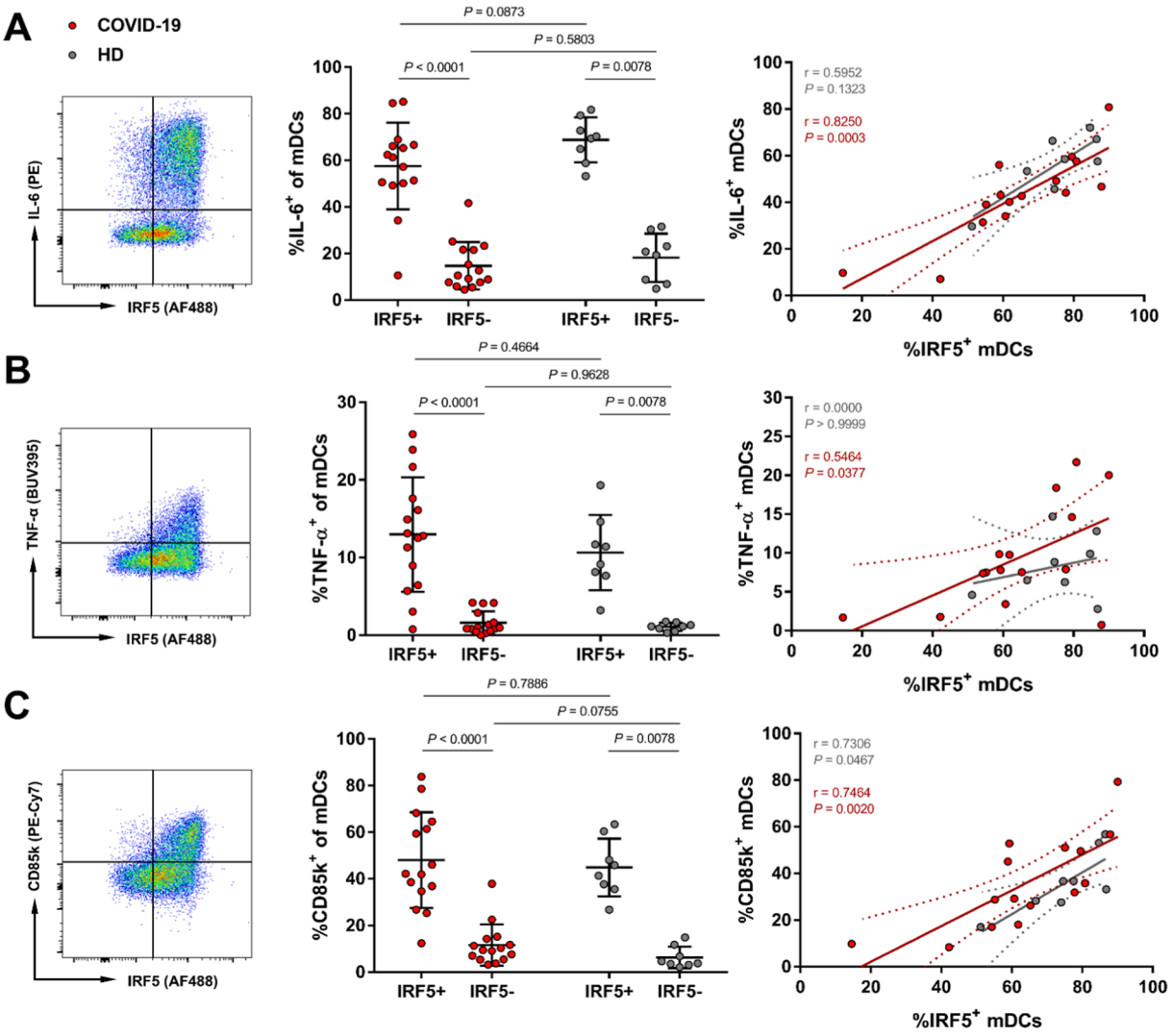

Fig. 4. Indicators for a role of IRF5 in the regulation of IL-6 and TNF-α production in mDCs. (**A**) IRF5+ mDCs more frequently produce IL-6, (**B**) TNF-α, and (**C**) express CD85k compared to their IRF5− counterparts upon TLR7 ligation and correlate with their frequencies. For comparison of IRF5+ and IRF5− cells within the COVID-19 group and the healthy donors, statistical significance was determined with a Wilcoxon matched-rank test. For all other analyses, a Mann-Whitney *U* test was performed. Spearman's rank correlation coefficient was calculated to determine correlations. Red dots indicate individuals with acute SARS-CoV-2 infection and grey dots show healthy donors.

mDCs of SARS-CoV-2 infected patients exhibited lower levels of IRF5 at baseline and both mDCs and pDCs tended to upregulate IRF5 less upon stimulation with TLR7 agonists compared with healthy individuals. However, DCs of SARS-CoV-2 infected patients produced comparable levels of inflammatory cytokines, including IFN-α, TNF-α, and IL-6. Our data provide strong indications for a potential role of IRF5 as a regulator of inflammatory cytokine responses in mDCs. These results identify IRF5 as a potential immunomodulatory target in COVID-19 and other inflammatory diseases.

Data availability statement

Data storage is performed by the *University Medical Center Hamburg-Eppendorf*. Data are available upon request from the corresponding author and can be shared after confirming that data will be used within the scope of the originally provided informed consent.

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Conflict of interest disclosure

The corresponding author (C.B.) states on behalf of all authors that there is no conflict of interest.

Author contributions

L.C., R.W., S.Kum., and C.B. performed research, collected and analyzed data; L.C., J. SzW., and C.B. designed and directed the research project; L.C. and C.B. reviewed the literature, and drafted the paper. S. K., A.W.L., M.A., M.M.A., and J.SzW. gave important input to the manuscript; T.T.B., S.Klu., S.S., and S.J. recruited study samples. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Leon Cords: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Robin Woost:** Investigation. **Silke Kummer:**

Investigation, Project administration. **Thomas T. Brehm:** Resources. **Stefan Kluge:** Resources. **Stefan Schmiedel:** Resources. **Sabine Jordan:** Resources. **Ansgar W. Lohse:** Resources. **Marcus Altfeld:** Validation, Writing – review & editing. **Marylyn M. Addo:** Resources, Writing – review & editing. **Julian Schulze zur Wiesch:** Conceptualization, Resources, Writing – review & editing, Supervision. **Claudia Beisel:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: 'Claudia Beisel reports financial support was provided by Deutsches Zentrum für Infektionsforschung (DZIF)'.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.cyto.2022.156109.

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